

Practitioner's Docket No. 49927

09/601371

## CHAPTER II

## Preliminary Classification:

Proposed Class:

Subclass:

NOTE: "All applicants are requested to include a preliminary classification on newly filed patent applications. The preliminary classification, preferably class and subclass designations, should be identified in the upper right-hand corner of the letter of transmittal accompanying the application papers, for example 'Proposed Class 2, subclass 129.'" M.P.E.P., § 601, 7th ed.

**TRANSMITTAL LETTER  
TO THE UNITED STATES ELECTED OFFICE (EO/US)**

**(ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)**

INTERNATIONAL APPLICATION NO. PCT/JP99/00414	INTERNATIONAL FILING DATE 1 February 1999	PRIORITY DATE CLAIMED 30 January 1998
TITLE OF INVENTION CYTOKINE INDUCERS COMPRISING M161Ag		
APPLICANT(S) Tsukasa SEYA and Misako MATSUMOTO		

**Box PCT**  
**Assistant Commissioner for Patents**  
**Washington D.C. 20231**  
**ATTENTION: EO/US**

**CERTIFICATION UNDER 37 C.F.R. § 1.10\***  
(Express Mail label number is mandatory.)  
(Express Mail certification is optional.)

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date July 30, 2000, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EK493920132US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Deanna M. Rivernider

(type or print name of person mailing paper)

Deanna M. Rivernider

Signature of person mailing paper

**WARNING:** Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. § 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.

**\*WARNING:** Each paper or fee filed by "Express Mail" **must** have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. § 1.10(b).  
"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

(Transmittal Letter to the United States Elected Office (EO/US) [13-18]—page 1 of 8)

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**NOTE:** To avoid abandonment of the application, the applicant shall furnish to the USPTO, not later than 20 months from the priority date: (1) a copy of the international application, unless it has been previously communicated by the International Bureau or unless it was originally filed in the USPTO; and (2) the basic national fee (see 37 C.F.R. § 1.492(a)). The 30-month time limit may not be extended. 37 C.F.R. § 1.495.

**WARNING:** Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 C.F.R. § 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing—See 37 C.F.R. § 1.8).

**NOTE:** Documents and fees must be clearly identified as a submission to enter the national state under 35 U.S.C. § 371 otherwise the submission will be considered as being made under 35 U.S.C. § 111. 37 C.F.R. § 1.494(f).

- I. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. § 371:
- a. ☒ This express request to immediately begin national examination procedures (35 U.S.C. § 371(f)).
  - b. ☒ The U.S. National Fee (35 U.S.C. § 371(c)(1)) and other fees (37 C.F.R. § 1.492) as indicated below:

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## 2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
<input type="checkbox"/>	TOTAL CLAIMS	13 -20=	0	× \$18.00=	\$
	INDEPENDENT CLAIMS	6 -3=	3	× \$78.00=	234.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable) + \$260.00				260.00
BASIC FEE**	<input type="checkbox"/> U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an international preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO: <input type="checkbox"/> and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 C.F.R. § 1.492(a)(4)) ..... \$96.00 <input type="checkbox"/> and the above requirements are not met (37 C.F.R. § 1.492(a)(1)) ..... \$670.00 <input checked="" type="checkbox"/> U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO: <input type="checkbox"/> has been paid (37 C.F.R. § 1.492(a)(2)) ..... \$690.00 <input type="checkbox"/> has not been paid (37 C.F.R. § 1.492(a)(3)) ..... \$970.00 <input checked="" type="checkbox"/> where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 C.F.R. § 1.492(a)(5)) ..... \$840.00				840.00
	Total of above Calculations				= 1,334.00
SMALL ENTITY	Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (note 37 C.F.R. § 1.9, 1.27, 1.28)				-
	Subtotal				1,334.00
	Total National Fee				\$ 1,334.00
	Fee for recording the enclosed assignment document \$40.00 (37 C.F.R. § 1.21(h)). (See Item 13 below). See attached "ASSIGNMENT COVER SHEET".				
TOTAL	Total Fees enclosed				\$ 1,334.00

\*See attached Preliminary Amendment Reducing the Number of Claims. **534 Rec'd PCT/PTC 31 JUL 2000**

- i. ☒ A check in the amount of 1,334.00 to cover the above fees is enclosed.
- ii. ☐ Please charge Account No. \_\_\_\_\_ in the amount of \$ \_\_\_\_\_.  
A duplicate copy of this sheet is enclosed.

**\*\*WARNING:** "To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: \* \* \* (2) the basic national fee (see § 1.492(a)). The 30-month time limit may not be extended." 37 C.F.R. § 1.495(b).

**WARNING:** If the translation of the international application and/or the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 C.F.R. § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(f) is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1.136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 O.G. 29 to 40.

3. ☒ A copy of the International application as filed (35 U.S.C. § 371(c)(2)):

**NOTE:** Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. "The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47.1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date." Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

- a. ☒ is transmitted herewith.
- b. ☐ is not required, as the application was filed with the United States Receiving Office.
- c. ☐ has been transmitted
- i. ☐ by the International Bureau.  
Date of mailing of the application (from form PCT/1B/308): \_\_\_\_\_
- ii. ☐ by applicant on \_\_\_\_\_  
Date

4. ☒ A translation of the International application into the English language (35 U.S.C. § 371(c)(2)):

- a. ☒ is transmitted herewith.
- b. ☐ is not required as the application was filed in English.
- c. ☐ was previously transmitted by applicant on \_\_\_\_\_  
Date
- d. ☐ will follow.

5. ☒ Amendments to the claims of the international application under PCT Article 19 (35 U.S.C. § 371(c)(3)):

NOTE: The Notice of January 7, 1993 points out that 37 C.F.R. § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that: "The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1.121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected." 1147 O.G. 29-40, at 36.

- a. ☐ are transmitted herewith.
- b. ☐ have been transmitted
  - i. ☐ by the International Bureau.  
Date of mailing of the amendment (from form PCT/1B/308): \_\_\_\_\_
  - ii. ☐ by applicant on (date) \_\_\_\_\_  
Date
- c. ☒ have not been transmitted as
  - i. ☒ applicant chose not to make amendments under PCT Article 19.  
Date of mailing of Search Report (from form PCT/ISA/210.): 20/04/99
  - ii. ☐ the time limit for the submission of amendments has not yet expired.  
The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. ☐ A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. § 371(c)(3)):
- a. ☐ is transmitted herewith.
  - b. ☐ is not required as the amendments were made in the English language.
  - c. ☒ has not been transmitted for reasons indicated at point 5(c) above.

7. ☒ A copy of the international examination report (PCT/IPEA/409)
- ☒ is transmitted herewith.
  - ☐ is not required as the application was filed with the United States Receiving Office.

8. ☒ Annex(es) to the international preliminary examination report
- a. ☒ is/are transmitted herewith.
  - b. ☐ is/are not required as the application was filed with the United States Receiving Office.

9. ☐ A translation of the annexes to the international preliminary examination report
- a. ☐ is transmitted herewith.
  - b. ☐ is not required as the annexes are in the English language.

10. ☒ An oath or declaration of the inventor (35 U.S.C. § 371(c)(4)) complying with 35 U.S.C. § 115
- ☐ was previously submitted by applicant on \_\_\_\_\_  
Date
  - ☐ is submitted herewith, and such oath or declaration
    - ☐ is attached to the application.
    - ☐ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. § 1.70.
  - ☒ will follow.

II. Other document(s) or information included:

11. ☒ An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):
- ☒ is transmitted herewith.
  - ☐ has been transmitted by the International Bureau.  
Date of mailing (from form PCT/IB/308): \_\_\_\_\_
  - ☐ is not required, as the application was searched by the United States International Searching Authority.
  - ☐ will be transmitted promptly upon request.
  - ☐ has been submitted by applicant on \_\_\_\_\_  
Date
12. ☒ An Information Disclosure Statement under 37 C.F.R. §§ 1.97 and 1.98:
- ☒ is transmitted herewith.  
Also transmitted herewith is/are:
    - ☒ Form PTO-1449 (PTO/SB/08A and 08B).
    - ☒ Copies of citations listed.
  - ☐ will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. § 371(c).
  - ☐ was previously submitted by applicant on \_\_\_\_\_  
Date
13. ☐ An assignment document is transmitted herewith for recording.  
A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

14. ☒ Additional documents:

- a. ☒ Copy of request (PCT/RO/101)
- b. ☒ International Publication No. W099/38523
- i. ☒ Specification, claims and drawing
- ii. ☐ Front page only
- c. ☐ Preliminary amendment (37 C.F.R. § 1.121)
- d. ☒ Other

PCT/IB/304, PCT/IB/332, PCT/IB/308, PCT/ISA/220

PCT/IPEA/416, PCT/IPEA/408, PCT/IPEA/401

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15. ☒ The above checked items are being transmitted

- a. ☒ before 30 months from any claimed priority date.
- b. ☐ after 30 months.

16. ☐ Certain requirements under 35 U.S.C. § 371 were previously submitted by the applicant on \_\_\_\_\_, namely:

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**AUTHORIZATION TO CHARGE ADDITIONAL FEES**

**WARNING:** Accurately count claims, especially multiple dependant claims, to avoid unexpected high charges if extra claims are authorized.

**NOTE:** "A written request may be submitted in an application that is an authorization to treat any concurrent or future reply, requiring a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. An authorization to charge all required fees, fees under § 1.17, or all required extension of time fees will be treated as a constructive petition for an extension of time in any concurrent or future reply requiring a petition for an extension of time under this paragraph for its timely submission. Submission of the fee set forth in § 1.17(a) will also be treated as a constructive petition for an extension of time in any concurrent reply requiring a petition for an extension of time under this paragraph for its timely submission." 37 C.F.R. § 1.136(a)(3).

**NOTE:** "Amounts of twenty-five dollars or less will not be returned unless specifically requested within a reasonable time, nor will the payer be notified of such amounts; amounts over twenty-five dollars may be returned by check or, if requested, by credit to a deposit account." 37 C.F.R. § 1.26(a).

- ☒ The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 04-1105.

☒ 37 C.F.R. § 1.492(a)(1), (2), (3), and (4) (filing fees)

**WARNING:** Because failure to pay the national fee within 30 months without extension (37 C.F.R. § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.

☒ 37 C.F.R. § 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 C.F.R. § 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

☒ 37 C.F.R. § 1.17 (application processing fees)

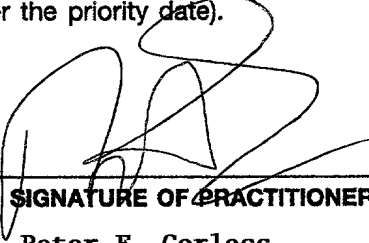
☒ 37 C.F.R. § 1.17(a)(1)-(5) (extension fees pursuant to § 1.136(a).

☐ 37 C.F.R. § 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. § 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 C.F.R. § 1.311(b).

NOTE: 37 C.F.R. § 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee." From the wording of 37 C.F.R. § 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

☐ 37 C.F.R. § 1.492(e) and (f) (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).



SIGNATURE OF PRACTITIONER

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CYTOKINE INDUCERS COMPRISING M161AgTechnical Field

This invention relates to cytokine inducers comprising protein M161Ag, immunomodulators and immunotherapeutic agents.

Background Arts

M161Ag is a membrane protein which is contained in cells latently infected with *Mycoplasma fermentans* such as a human myelocytic leukemia cell line P39(+), and has functions such as activation of the alternative pathway and adsorption of the complement C3. Isolation and purification of this protein and preparation of monoclonal antibody have already been reported [Matsumoto et al., J. Exp. Med. 181, 115-125 (1995)]. Further, the primary structure has almost been reported [Nature Med., 3: 1266-1270 (1997)](Japanese Patent Unexamined Publication No. Hei 9-157295).

*Mycoplasma fermentans* (*M. fermentans*) is an intracellular parasitic bacterium, which appears to positive under the disease states of immunosuppressive conditions, for example, HIV infected patients, patients with cancer such as leukemia and myeloma and patients with aplastic anemia. Main parasitic host is identified in vitro as human tumor cell strain. It is known that in a positive case of *M. fermentans*, M161Ag is essentially positive.

In the report by the another group, Mycoplasma is a cause of lymphopenia and is a cofactor of AIDS crisis. Further, *M. fermentans* has been suggested to induce cytokines as a result of stimulation of leukocytes system

in vitro. These phenomena are not generated by an action of other Mycoplasma species and are observed in *M. fermentans*. Consequently, a gene product specific to *M. fermentans* might instruct lymphopenia and cytokine induction. However, the fact that what substance may be involved in those actions is unknown. We have identified a substance, which was involved in these biological activities of *M. fermentans*, by an assay using purified authentic sample, and demonstrated the range of the biological activities, thereby completed the present invention.

An object of the present invention is to provide cytokine inducers, immunomodulators and immunotherapeutic agents comprising M161Ag by applying physiological activity and cytokine inducing activity of M161Ag, and to make good use of treatment for various immunological diseases.

#### Disclosure of Invention

We have succeeded, using purified authentic sample of M161Ag, to induce effectively the inflammatory cytokines such as IL-1 $\beta$ , TNF- $\alpha$  and IL-6 and the lymphocyte-activating cytokines such as IL-10 and IL-12 by stimulating immune competent cells such as monocytes and lymphocytes, and to remove infected cells as a result of apoptosis, and completed the present invention.

The present invention relates to cytokine inducers, immunomodulators or remedies for various immunological diseases comprising the protein M161Ag or gene recombination products thereof.

In addition, the present invention relates to therapeutic method for diseases caused by cytokine deficiency, immunological diseases and immune

diseases comprising administering therapeutically sufficient amounts of the protein M161Ag or gene recombination products thereof. Further, the present invention relates to use of the protein M161Ag or gene recombination products thereof for production of cytokine inducers, immunomodulators or remedies for immunological diseases.

DNA of M161Ag of the present invention can be obtained as genomic DNA from human myelocytic leukemia cell line P39 and human fibroblast cell line W138, in which *M. fermentans* is infected and proliferated. Since these DNAs contain five codons of TGA (termination codon in *E. coli* and human; and tryptophan in *Mycoplasma*), these codons are replaced by TGG, and the resultant sequence is inserted into the expression vector of *E. coli* having His-tag such as pET, then is forcibly expressed in cells. The thus obtained large amount of gene recombination products is purified by nickel Sepharose column. The obtained sample is confirmed as *E. coli* derived pyrogen-free.

Thus purified M161Ag has 45kDa of molecular weight and is bound with lipid such as palmitic acid in N-terminal cysteine. When M161Ag is mixed with cells, a part of M161Ag is incorporated into cells by an action of this lipid. When M161Ag is mixed with peripheral blood, monocytes and monocyte system cells in vitro, the inflammatory cytokines derived from macrophage such as IL-1 $\beta$ , TNF- $\alpha$  and IL-6, and cytokines having modulator activity of lymphocytes such as IL-10 and IL-12 can be identified in the cultured supernatant solution within 24 hours. From comparison of an activity of M161Ag with that of lipopolysaccharide (LPS), in case of using identical weight (such as 10 ng) of these substances, all of the above cytokines are found to be more potentially induced by M161Ag than by LPS. Monocytes used in

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this experiment are prepared by the method of Karp et al. using elutriation system (Beckman) and have purification above 95%. Consequently, it can be concluded that these cytokines were induced by direct stimulation of M161Ag to monocytes.

It can be proved that M161Ag is a bioactive substance, which potentially induces human cytokines, derived from Mycoplasma. In addition, it has been elucidated that M161Ag activated human fundamental immune system (innate immunity) through this action and improved host immunity in the immunosuppressive condition, further resulted the infected cells such as lymphocytes to apoptotic death, and suppressed disease state caused by excess immune activation such as allergy.

As explained hereinabove, it is proved that M161Ag induces cytokines as a modulator in various immune system through the monocyte system.

#### Brief Description of Drawing

Fig. 1 indicates inducing action of the protein M161Ag of the present invention on IL-1 $\beta$ , TNF- $\alpha$  and IL-6 in the monocyte cell THP-I. In Fig. 1, the left columns show data from cell lysates and the right columns show data in the conditioned medium.

Fig. 2 indicates inducing action of the protein M161Ag of the present invention on IL-1 $\beta$ , TNF- $\alpha$  and IL-6 in the purified blood monocytes. In Fig. 2, the left columns show data from cell lysates and the right columns show data in the conditioned medium.

Fig. 3 indicates inducing action of the protein M161Ag of the present invention on IL-10 (Fig. 3, left) and IL-12 (Fig. 3, right).

### Best Mode of Carrying Out the Invention

The cytokines induced by the protein M161Ag of the present invention are known to have various bioactivities. For example, IL-1 is known to have activities of T cell activation, neutrophil activation, stimulation of antitumor activity, proliferation of fibroblasts and increase of ACTH and GH.  $\text{TNF-}\alpha$  is a factor for involving in proliferation and differentiation of cells, and especially is a factor having tumor necrotizing action. It is reported also to increase production of prostaglandins and platelet activating factor, further to have antiviral action.

IL-6 has actions to stimulate differentiation of B cells to antibody production cells as well as to stimulate differentiation and proliferation of T cell and macrophage. IL-10 is a factor having an action to proliferate T cell. IL-12 is a factor having actions to activate cytotoxic T cell (CTL) and NK cell.

M161Ag can be applied its cytokine inducing activity for treatment of various immunological diseases and cancers.

Since the active component of the present invention is to activate human innate immunity system, it is useful for remedies for various immunological diseases involved in the innate immunity system. Examples of immunological diseases are, for example, allergic diseases and autoimmune diseases.

The protein M161Ag of the present invention can be the extract of cells such as cell line P39(+), and is also able to be the expression product (gene recombination product) by using DNA with various host cells as disclosed in Japanese Patent Unexamined Publication No. Hei 9-157295.

In a sequence of the protein M161Ag of the present invention, one or more amino acids may be deleted; one or more amino acids may be substituted to the other amino acids; or one or more amino acids may be added, within maintaining its activity.

The M161Ag of the present invention can be used in the form of protein itself, in addition to that, it can be used in the form that the protein is modified with fatty acid or other substances in the N-terminal or the suitable position in the amino acid sequence, or the protein is modified by binding with specific antibody to cells such as cancer cells.

Amount of administration of the protein M161Ag of the present invention as an active ingredient depends on conditions of patients and types of diseases, and is usually 0.01 - 100 ng/kg/day, preferably 0.1 - 10 ng/kg/day for 1 - 3 times/day.

Routes of administration are preferably intravenous injection and subcutaneous injection, however there is no specific limitation if administered parenterally such as using sublingual tablets and suppositories.

Preparations prepared by conventional protein formulations can be used in the administration form. Emulsion such as liposome preparation can also be used.

### Examples

The present invention is explained more concretely by the following examples, but is not construed as limiting by these examples.

### Example 1

M161Ag, 6 ng and 12 ng, respectively, was added to the monocyte cell system line THP-1 ( $10^6$ ). Culture supernatant was collected after 24 hours and various cytokines were quantitatively assayed by sandwich ELISA. Fig. 1 shows results of assay as well as comparative data of using LPS.

### Example 2

M161Ag, 2.4 ng and 12 ng, respectively, was added to the purified peripheral blood monocytes ( $10^6$ ). Culture supernatant was collected after 24 hours and various cytokines were quantitatively assayed by sandwich ELISA. Fig. 2 shows results of assay as well as comparative data of using LPS.

### Example 3

M161Ag, 2.4 ng and 12 ng, respectively, was added to the purified peripheral blood monocytes ( $10^6$ ). After 24 hours, IL-10 and IL-12 were quantitatively assayed. Results are shown in Fig. 3.

### Industrial Applicability

The present invention provides pharmaceutical agent comprising novel bioactivity of membrane protein M161Ag of *Mycoplasma fermentans*. M161Ag shows potential cytokine inducing activities and can be applied as immunomodulators for allergic and immunosuppressive conditions.

## CLAIMS

1. Cytokine inducers comprising a protein M161Ag or gene recombination products thereof.
2. The cytokine inducers according to claims 1 wherein induced cytokines are IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-10, IL-12 and/or INF- $\gamma$ .
3. The cytokine inducers according to claim 1 or 2 wherein the cytokine inducers are used as immunomodulators.
4. The cytokine inducers according to claim 3 wherein the immunomodulators are used as remedies for immunological diseases.
5. The cytokine inducers according to any one of claims 1 - 4 wherein the protein M161Ag or gene recombination products thereof are acylated with fatty acid in N-terminal thereof.
6. A remedy for immunological diseases comprising protein M161Ag or gene recombination products thereof.
7. A therapeutic method for diseases caused by cytokine deficiency comprising administering therapeutically sufficient amount of protein M161Ag or gene recombination products thereof.
8. A therapeutic method for immunological diseases comprising administering therapeutically sufficient amount of protein M161Ag or gene recombination products thereof.
9. Use of protein M161Ag or gene recombination products thereof for production of cytokine inducers.
10. Use of protein M161Ag or gene recombination products thereof for production of remedies for immunological diseases.



# ABSTRACT

Cytokine inducers comprising a membrane protein M161Ag which is contained in cells latently infected with *Mycoplasma fermentans* such as a human myelocytic leukemia cell line P39(+). These cytokine inducers comprising the membrane protein M161Ag or gene recombination products thereof are useful as immunomodulators or remedies for various immunological diseases.

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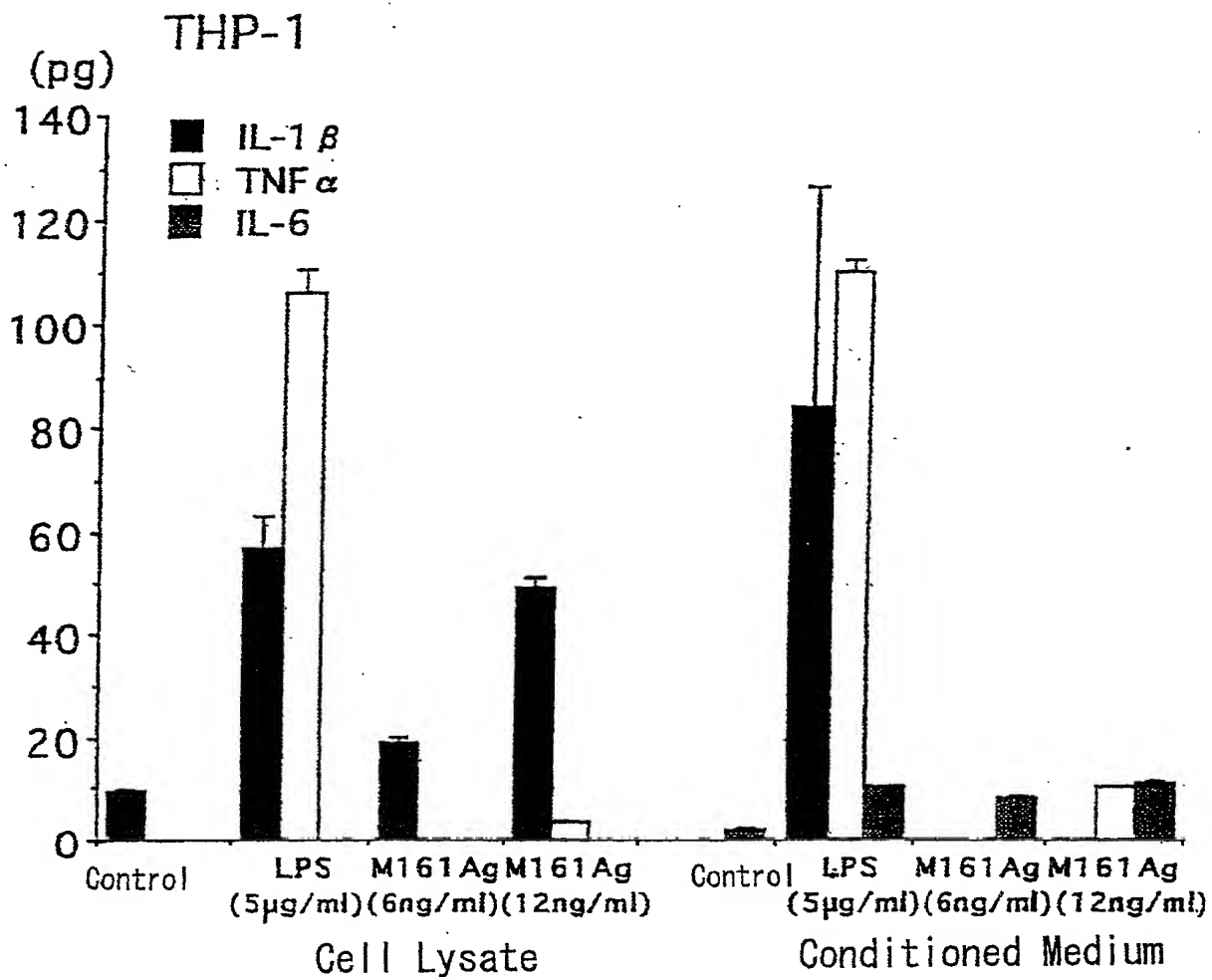


Figure 1

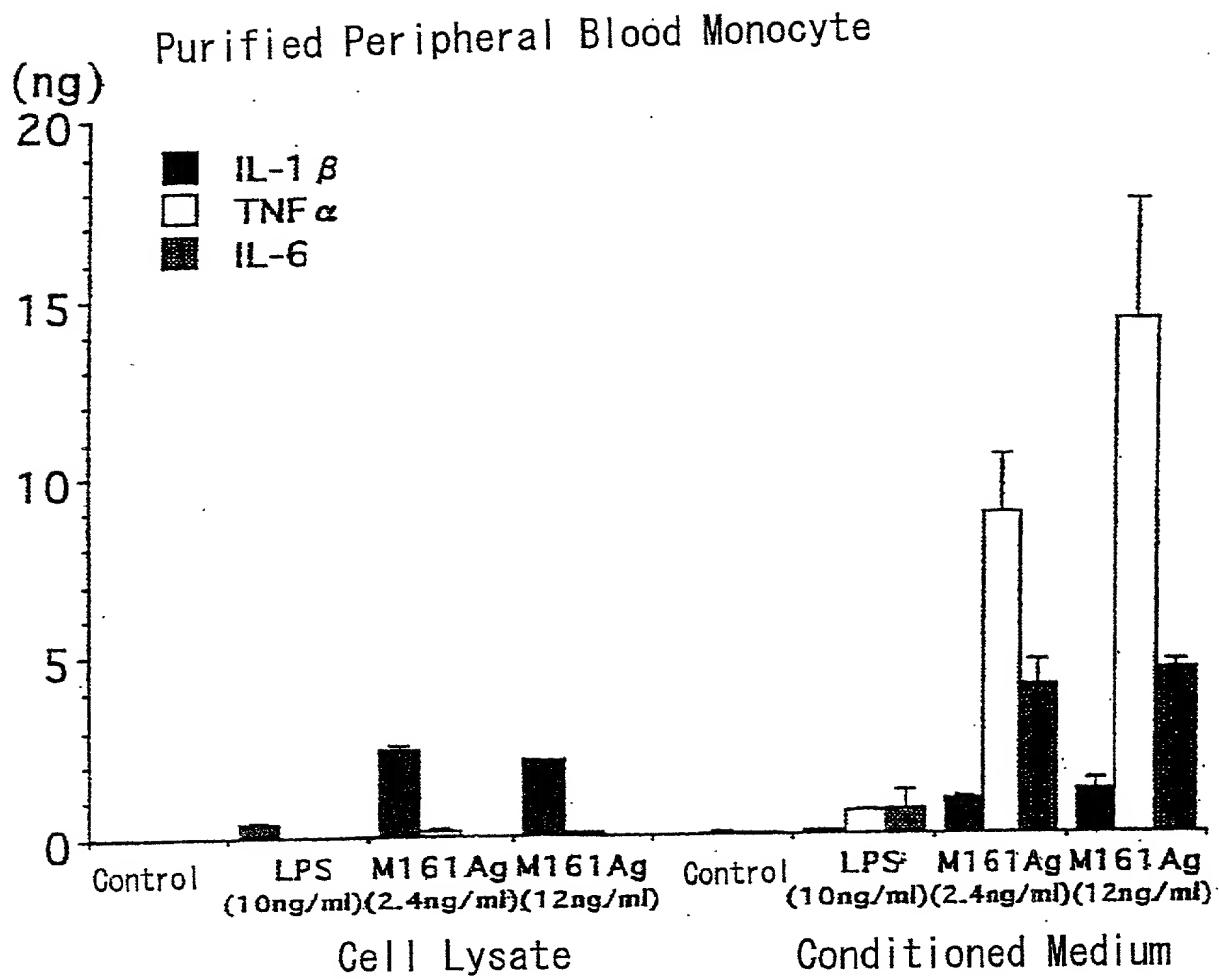


Figure 2

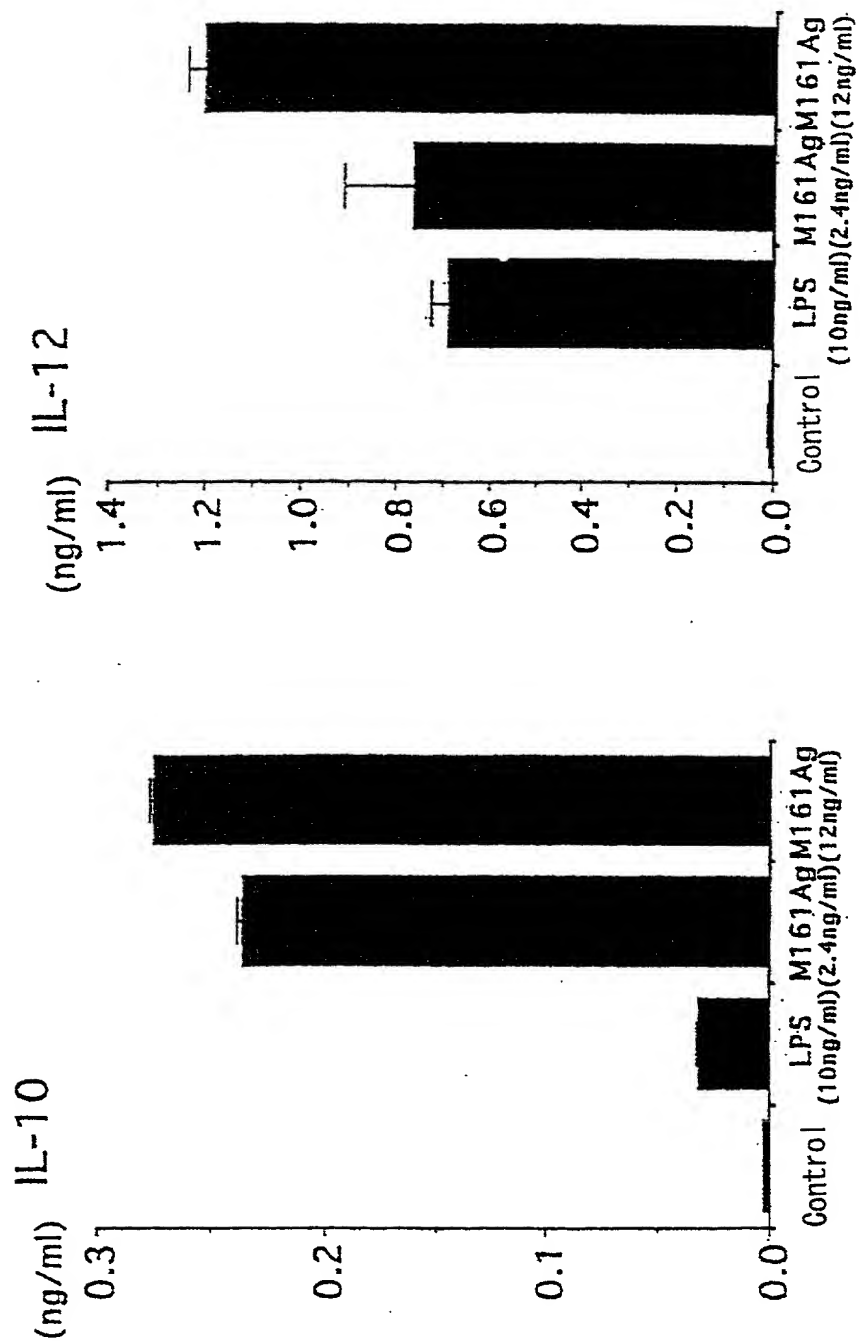


Figure 3

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

### CYTOKINE INDUCERS COMPRISING M161Ag

the specification of which

(check one)

[ ] is attached hereto.  
[X] was filed on July 30, 2000 as United States Application No. or PCT  
Application No. 09/601,371  
and was amended on \_\_\_\_\_  
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

<u>10-32384/1998</u>	<u>Japan</u>	<u>January 30, 1998</u>	[ ]
(Number)	(Country)	(Day/Month/Year Filed)	

\_\_\_\_\_  
(Number) (Country) (Day/Month/Year Filed) [ ]

\_\_\_\_\_  
(Number) (Country) (Day/Month/Year Filed) [ ]

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

_____	_____
(Application Serial No.)	(Filing Date)
_____	_____
(Application Serial No.)	(Filing Date)
_____	_____
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. Section 120 of the United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark office all information known to me to be material to patentability as defined in Title 37, C.F.C., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

_____	_____	_____
PCT/JP99/00414	February 1, 1999	Pending
(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)
_____	_____	_____
(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)
_____	_____	_____
(Application Serial No.)	(Filing Date)	(Status)
		(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)


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